

36. (New) The method according to Claim 31, wherein said complex offers two available valencies.

B1 37. (New) The immobilized metal ion affinity chromatography complex according to Claim 36, wherein said two available valencies form strong, reversible complexes with adjacent histidine residues on the surface of said protein. --

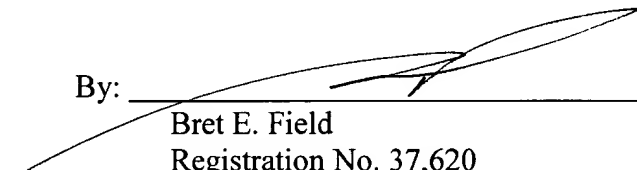
**REMARKS UNDER 37 CFR § 1.111**

Newly presented Claims 24-37 find support throughout the specification and originally pending claims. As such, there entry and examination by the Examiner is respectfully requested.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number CLON37DIV.

Respectfully submitted,  
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Date: 3.12.02

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the claims:**

Please add the following new claims:

- 24. (New) An immobilized metal ion affinity chromatography complex comprising:
- (a) an aspartate metal chelating ligand; and
  - (b) a transition metal ion complexed to said aspartate metal chelating ligand.
25. (New) The immobilized metal ion affinity chromatography complex according to Claim 24, wherein said aspartate ligand is a tetradentate ligand.
26. (New) The immobilized metal ion affinity chromatography complex according to Claim 24, wherein said transition metal is complexed to said ligand in octahedral geometry.
27. (New) The immobilized metal ion affinity chromatography complex according to Claim 24, wherein said transition metal ion is selected from the group consisting of  $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ .
28. (New) The immobilized metal ion affinity chromatography complex according to Claim 27, wherein said transition metal ion is  $\text{Co}^{2+}$ .
29. (New) The immobilized metal ion affinity chromatography complex according to Claim 24, wherein said complex offers two available valencies.
30. (New) The immobilized metal ion affinity chromatography complex according to Claim 29, wherein said two available valencies form strong, reversible complexes with adjacent histidine residues on the surface of a protein.

31. (New) An immobilized metal ion affinity chromatography purification method for purification of recombinant proteins, said method comprising:
- (a) providing an immobilized metal ion affinity chromatography complex comprising:
    - (i) an aspartate metal chelating ligand; and
    - (ii) a transition metal ion complexed to said aspartate metal chelating ligand;
  - (b) loading a mixture of cell lysate comprising a recombinant protein having a polyhistidine tail to bind with said complex; and
  - (c) eluting said recombinant protein with a suitable elutant to obtain a purified recombinant protein.
32. (New) The method according to Claim 31, wherein said aspartate metal chelating ligand is a tetradentate ligand.
33. (New) The method according to Claim 31, wherein said transition metal is complexed to said ligand in octahedral geometry.
34. (New) The method according to Claim 31, wherein said transition metal ion is selected from the group consisting of  $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ .
35. (New) The method according to Claim 34, wherein said transition metal ion is  $\text{Co}^{2+}$ .
36. (New) The method according to Claim 31, wherein said complex offers two available valencies.
37. (New) The immobilized metal ion affinity chromatography complex according to Claim 36, wherein said two available valencies form strong, reversible complexes with adjacent histidine residues on the surface of said protein. --